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# Reactivation of Herpes Simplex Virus Type 1 in Patients With Bell's Palsy

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Reactivation of herpes simplex virus type 1 (HSV-1) has been implicated in the pathogenesis of idiopathic peripheral facial palsy (Bell's palsy). The present study used the polymerase chain reaction (PCR) to analyze the saliva of patients with Bell's palsy for the presence of shed HSV-1. The study involved 47 patients with Bell's palsy, 24 patients with Ramsay Hunt syndrome, and 16 healthy HSV-seropositive volunteers. HSV-1 DNA was not detected in the saliva samples from HSV-seronegative patients. The prevalence of shed HSV-1 in patients with Bell's palsy (50%) was significantly higher than that in healthy volunteers (19%, $p<0.05$). When saliva samples were tested within 7 days after the onset of palsy, the prevalence of shed HSV-1 in patients with Bell's palsy (40%) was significantly higher than that in patients with Ramsay Hunt syndrome (7%, $p<0.05$). Furthermore, HSV-1 usually became undetectable by the second week after the onset of Bell's palsy when HSV-1 was detected during the acute phase of the disease. These findings strongly suggest that reactivation of HSV-1 is involved in the pathogenesis Bell's palsy, and indicate that PCR is a useful tool for early diagnosis of HSV-1 reactivation in patients with Bell's palsy. ***J. Med. Virol. 54:162–166, 1998.*** © 1998 Wiley-Liss, Inc.

**KEY WORDS:** saliva; PCR; Ramsay Hunt syndrome

## INTRODUCTION

Various disorders, such as otitis media, temporal bone fracture, and carcinoma of the parotid gland, cause peripheral facial paralysis. Varicella-zoster virus affects the facial nerve associated with or without herpetic rash; such cases are known as Ramsay Hunt syndrome. Most patients with acute peripheral facial palsy, however, are given a diagnosis of "idiopathic," or Bell's palsy by the exclusion of known etiology.

Viral infection is most likely involved in the pathogenesis of Bell's palsy, and herpes simplex virus (HSV) has been extensively investigated [Morgan, 1992; Spruance, 1994]. Since McCormick [1972] suggested that reactivation of HSV causes inflammation and edema in the bony fallopian canal and results in peripheral facial palsy, several virological [Mulkens et al., 1980], serological [Adour et al., 1975; Vahlne et al., 1981], and molecular biological [Murakami et al., 1996] studies have attempted to clarify this hypothesis. Although supporting evidence has increased [Baringer, 1996], HSV-1 reactivation remains to be confirmed in Bell's palsy.

After primary infection, HSV-1 establishes latency in the trigeminal [Baringer & Swoveland, 1973] and the geniculate [Furuta et al., 1992a; Takasu et al., 1992] ganglia. Certain stimuli, such as stress or trauma, can cause viral reactivation, after which the virus migrates along the nerve to the epithelium. If the reactivation of HSV-1 in the geniculate or trigeminal ganglia causes acute facial palsy, HSV-1 is shed into the saliva via either the chorda tympani nerve or the trigeminal nerve, and should be detectable at the onset of palsy. Only a few investigators have reported on the isolation of HSV-1 from the oropharynx in patients with Bell's palsy [Djupesland et al., 1976; Vahlne et al., 1981], and no study has compared the frequency of HSV-1 isolation between patients with Bell's palsy and control subjects. To gain further insight into the relationship between Bell's palsy and reactivation of HSV-1, the prevalence of shed HSV-1 into saliva in patients with Bell's palsy or Ramsay Hunt syndrome, and in healthy HSV-seropositive volunteers was tested by using a sensitive nested-polymerase chain reaction (PCR) method.

## MATERIALS AND METHODS

### Control viral DNA

DNA from HSV-1 strains K192 and K193, HSV-2 strains 20 and 27, varicella-zoster virus strain H-N3,

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and human cytomegalovirus strain KH were obtained from Dr. R. Hondo (Nippon Veterinary and Animal Science University, Koganei, Japan). DNA extracted from Raji cells (containing Epstein-Barr virus DNA) and the DNA of human herpesvirus-6 strain OK were provided by Dr. H. Kikuta (Hokkaido University, Sapporo, Japan).

### Specimens from patients and healthy volunteers

Informed consent was obtained from all patients and healthy volunteers. Forty-seven patients with Bell's palsy were examined. These patients visited the hospital within 2 weeks after the onset of the disease. Facial palsy developed in one patient after dental treatment, exhibiting herpes labialis on the affected side. In total, 162 saliva samples were collected from 42 HSV-seropositive patients with Bell's palsy. Twenty-four patients with Ramsay Hunt syndrome were also included in this study. Forty-nine saliva samples were obtained from 16 HSV-seropositive patients with Ramsay Hunt syndrome. Forty-eight control samples were collected from 16 healthy volunteers who had been examined and found to be positive for anti-HSV IgG antibody. The saliva supernate was stored at −80°C until use.

### Polymerase chain reaction

Saliva samples were digested with proteinase K (0.1 mg/ml, Boehringer Mannheim, Germany), and total DNA was prepared by phenol/chloroform extraction and ethanol precipitation. Two pairs of primers that are specific for the thymidine kinase gene of HSV-1 were used to detect HSV-1 DNA, using a nested-PCR method. The primers used for the first PCR were HSV-TK 3 (5′-ATGGTCCAGACCCACGTCAC-3′) and HSV-TK 4 (5′-AACACCCGTGCGTTTTATTC-3′), according to the sequence data [McGeoch et al., 1988]. Two μl of the first PCR product was re-amplified by PCR using HSV-TK 1 (5′-ATACCGACGATATGCGACCT-3′) and HSV-TK 2 (5′-TTATTGCCGTCATAGCGCGG-3′) as the inner primer pair [Lynas et al., 1989]. PCR amplification was performed using a 100-μl reaction mixture consisting of 10 μl of 10× PCR buffer (Perkin Elmer, Norwalk, U.S.A.), a deoxynucleotide triphosphate mixture (dATP, dGTP, dCTP, and dTTP at a final concentration of 125 μM), 0.5 units of Taq DNA polymerase (Perkin Elmer), and 20 pmol of each primer. Reagents were cycled 30 times on a DNA thermal cycler (Temp Tronic, Thermolyne, Dubuque, U.S.A.), each cycle consisting of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1 min.

### Detection of amplified product

One-tenth of the final reaction mixture was electrophoresed on 2% agarose gels containing ethidium bromide (0.7 μg/ml). An amplified band (110 bp) was visible on the gel in samples containing HSV-1 DNA. To confirm that the amplified DNA originated from HSV-1 DNA, the DNA was transferred to nylon membranes (Hybond N; Amersham, Amersham, U.K.). The amplified DNA was identified using a Southern blot hybridization and chemiluminescence method with a digoxigenin-labeled internal oligonucleotide probe (5′-digoxigenin-GGAGGCTAACTGAAACACGGAAG-GAGACAA-3′), as described previously [Furuta et al., 1992b].

### Serological assays

Sera were taken from all patients at their initial visit. In most patients, paired sera were obtained 2 or 3 weeks later. Anti-HSV IgG and IgM antibody titers were measured using an enzyme-linked immunosorbent assay (Enzygnost Anti-HSV/IgG and IgM, Behringwerke, Marburg, Germany) according to the manufacturer's instructions. Either significant changes (over two-fold) in anti-HSV IgG antibody titer or detection of increased anti-HSV IgM antibody titer (greater than 0.1 ELISA value) was considered to indicate recent HSV infection.

## RESULTS

### Sensitivity and specificity of the nested-PCR

The sensitivity of the nested-PCR method was assessed by using a dilution series of the BamHI-Q restriction fragment of HSV-1 DNA, in the presence of DNA extracted from the saliva samples of an HSV-seronegative volunteer. An amplified band of 110 bp was detectable on the ethidium bromide-stained agarose gel in samples with concentrations of HSV-1 DNA fragment as low as 0.1 fg (equivalent to approximately 2 copies of the HSV-1 genome). The DNAs of HSV-1 strains K192 and K193 produced positive PCR results using agarose gel analysis, and the amplified bands were hybridized with the internal oligonucleotide probe by Southern blot hybridization. Two amplified DNA bands, 110 bp and approximately 300 bp, were detected by PCR when the DNAs from HSV-2 strains 20 and 27 were used. The longer fragment was more intense than the 110-bp fragment, and we were able to distinguish HSV-1 DNA from HSV-2 DNA. No amplified fragments were observed on the agarose gel when the DNAs of varicella-zoster virus, human cytomegalovirus, Epstein-Barr virus, or human herpesvirus-6 were used. Thus, the nested-PCR method is sensitive and specific for the detection of HSV-1 DNA (data not shown).

### Serological assays

Anti-HSV IgG antibody was detected in 42 of 47 (89%) patients with Bell's palsy and 16 of 24 (67%) patients with Ramsay Hunt syndrome. The percentage of HSV-seropositive patients with Bell's palsy was significantly higher than that with Ramsay Hunt syndrome ($p<0.05$, Fisher exact test). Either significant changes in anti-HSV IgG titer or detection of increased anti-HSV IgM antibody titer was observed in three patients with Bell's palsy; no such correlation, however, was observed in patients with Ramsay Hunt syndrome.

TABLE I. Shed HSV-1 into saliva of patients with facial palsy and of volunteers

| Disease | No. PCR-positive cases/No. seropositive cases tested | | |
|---|---|---|---|
| | Day 0[a]-7 | Day 8- | Total |
| Bell's palsy | 16/40 (40%)[b] | 8/34 (24%) | 21/42 (50%)[c] |
| Ramsay Hunt syndrome | 1/15 (7%)[b] | 3/12 (25%) | 4/16 (25%) |
| Healthy volunteers | | | 3/16 (19%)[c] |

[a]Day 0 is the day of appearance of facial palsy.
[b]$p = 0.022$, Fisher exact test.
[c]$p = 0.039$, Fisher exact test.

TABLE II. Detection of HSV-1 DNA in saliva samples by PCR

| Disease | No. PCR-positive saliva samples/No. tested | | |
|---|---|---|---|
| | Day 0-7 | Day 8- | Total |
| Bell's palsy | 20/75 (27%)[a][b] | 12/87 (14%)[b] | 32/162 (20%)[c] |
| Ramsay Hunt syndrome | 1/25 (4%)[a] | 5/24 (21%) | 6/49 (12%) |
| Healthy volunteers | | | 3/48 (6%)[c] |

[a]$p = 0.021$, Fisher exact test.
[b]$p = 0.040$, chi-square test.
[c]$p = 0.028$, Fisher exact test.

### HSV-1 DNA in healthy seropositive volunteers

From each HSV-seropositive volunteer, three saliva samples were collected on three different days. HSV-1 DNA was detected in 3 of 16 (19%) volunteers on one occasion; therefore, the frequency of HSV-1-positive saliva samples in healthy volunteers was 6% (Tables I and II).

### HSV-1 DNA in patients with facial palsy

HSV-1 DNA was not detected in any of the saliva samples from five HSV-seronegative patients with Bell's palsy or from eight seronegative patients with Ramsay Hunt syndrome. HSV-1 DNA was detected on at least one occasion in 21 of 42 (50%) HSV-seropositive patients with Bell's palsy, and in 3 of 16 (19%) seropositive healthy volunteers. The difference between the two groups was statistically significant ($p<0.05$, Table I). The frequency of HSV-1-positive saliva samples in patients with Bell's palsy (20%) was also significantly higher than that in healthy volunteers (6%, $p<0.05$, Table II). The prevalence of HSV-1 DNA was higher in patients with Bell's palsy (50%) than in patients with Ramsay Hunt syndrome (25%), but the difference was not statistically significant ($p>0.05$, Table I). Shedding of HSV-1 into saliva was detected in 16 of 40 (40%) HSV-seropositive patients with Bell's palsy when saliva was tested within 7 days after onset of palsy; shed HSV-1, however, was detected in only 1 of 15 (7%) seropositive patients with Ramsay Hunt syndrome who were tested within 7 days after the onset of palsy. The difference between the two groups was statistically significant ($p<0.05$, Table I). The frequency of HSV-1-positive saliva samples obtained within 7 days after the onset of Bell's palsy (27%) was significantly higher than that obtained 8 days or more after onset (14%, $p<0.05$, Table II). In contrast, the frequency of HSV-1-positive saliva samples obtained 8 days or more after onset of Ramsay Hunt syndrome (21%) was higher than that obtained within the first 7 days after onset (4%, Table II). In all but three patients with Bell's palsy who shed HSV-1 into saliva within 7 days after the onset, HSV-1 DNA was detected at the first PCR assay, and HSV-1 usually disappeared by the second week after onset (case B 1 to case B 16, Fig. 1). HSV-1 DNA was detected in the saliva sample obtained after dental treatment of one patient displaying herpes labialis at the initial visit (case B 5, Fig. 1).

## DISCUSSION

This study shows that the prevalence of shed HSV-1 into saliva in patients with Bell's palsy is higher than that in controls. The higher frequency of HSV-seropositive patients with Bell's palsy is consistent with the results of previous studies [Adour et al., 1975; Vahlne et al., 1981]; however, only a few reports have described the isolation of HSV from oropharynx in patients with Bell's palsy, and no investigator has used PCR to detect shed HSV. Djupesland et al. [1976], by using a co-cultivation method, isolated herpesvirus hominis from the nasopharynx in 2 of 51 patients with Bell's palsy, although these isolations were not confirmed serologically. In contrast, Vahlne et al. [1981] were not able to isolate any virus from 36 patients with Bell's palsy. Thus, the attempts to detect shed HSV-1 in patients with Bell's palsy have produced equivocal findings, probably due to the lack of sensitivity of the virus isolation method used.

In the present study, we analyzed saliva samples using a sensitive nested-PCR method, and were able to detect HSV-1 DNA in saliva samples obtained from patients with Bell's palsy or Ramsay Hunt syndrome, and from healthy volunteers who were tested positive for anti-HSV antibody. The virus-isolation method detected asymptomatic shedding of HSV-1 into saliva in 4.5% of healthy Japanese people [Kameyama et al., 1988]. Tateishi et al. [1994] reported that PCR was about two-fold more sensitive than virus isolation as a method for detection of shed HSV-1 into saliva. Therefore, the frequency of HSV-1-positive saliva samples in our healthy volunteers is consistent with the results of other investigations.

The isolation of HSV-1 in saliva samples from patients with Bell's palsy does not directly indicate that HSV-1 causes facial palsy, because HSV-1 has been also detected in saliva samples from healthy people. We compared the frequency of shed HSV-1 in patients with Bell's palsy with that of healthy volunteers, and found that the frequency of shed HSV-1 was significantly higher in patients with Bell's palsy. The data support the hypothesis that HSV-1 is implicated in the

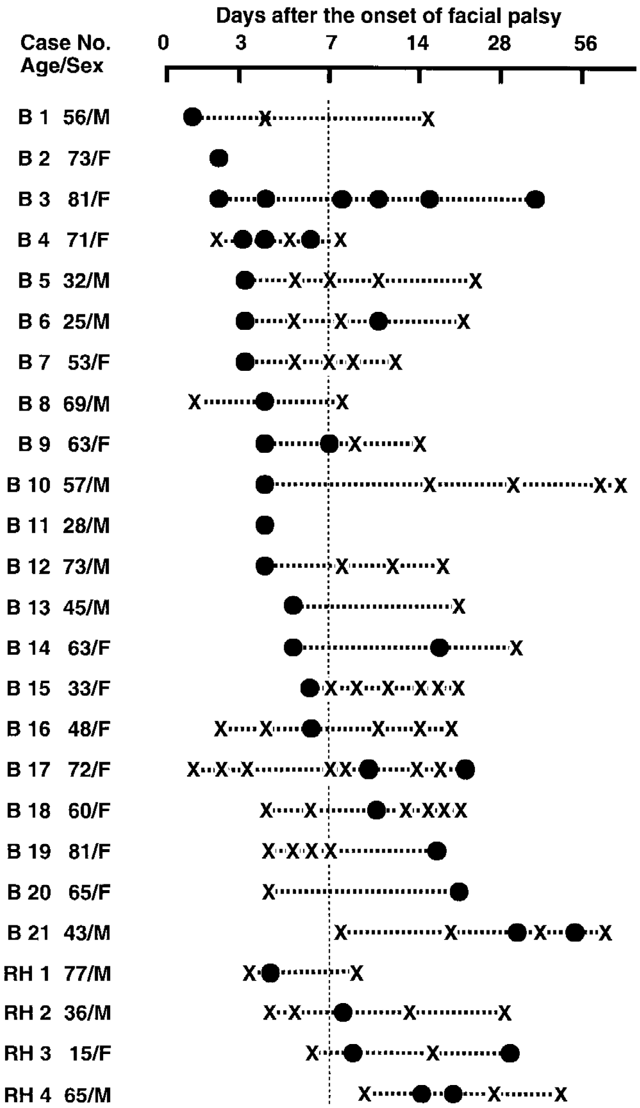


Fig. 1. Shedding of HSV-1 into saliva of 21 patients with Bell's palsy (B 1 to B 21) and 4 patients with Ramsay Hunt syndrome (RH 1 to RH 4). ●: HSV-1 DNA detected in the saliva sample by PCR. X: HSV-1 DNA not detected. Day 0 is the day of appearance of facial palsy. HSV-1 was detected within 7 days after the onset of palsy in cases B 1 to B 16 and RH 1. In cases B 17 to B 21 and RH 2 to RH 4, shed HSV-1 was detected in saliva samples obtained 8 days or more after onset of palsy.

pathogenesis of Bell's palsy. There was no significant difference, however, in the prevalence of HSV-1 DNA between patients with Bell's palsy and those with Ramsay Hunt syndrome; it is therefore possible that stress due to the facial palsy or steroid therapy causes reactivation of HSV-1 immediately after the onset of these diseases. Our data reveal differences in HSV-1 shedding for patients in the acute phases of Bell's palsy or Ramsay Hunt syndrome. In patients with Bell's palsy, shed HSV-1 was often detected within one week after the onset of palsy; yet for patients with Ramsay Hunt syndrome, reactivation of HSV-1 was rarely observed within one week after the onset of palsy. Although acyclovir was administered in some patients with Ramsay Hunt syndrome, all except two saliva samples were tested in the very acute phase of the disease before the initiation of anti-viral therapy. Therefore, the observed differences in HSV-1 shedding were not influenced by treatment regimens. Reactivation of HSV-1 during the acute phase of Bell's palsy may relate significantly to the onset of palsy, because HSV-1 usually becomes undetectable by the second week after onset; in addition, shed HSV-1 was rarely detected during the acute phase of Ramsay Hunt syndrome.

In most cases of Bell's palsy, HSV-1 shed into saliva was detected in patients without herpetic lesions. One exception was a patient with Bell's palsy whose facial palsy occurred on the herpes labialis-affected side following dental treatment. Since dental therapy produces clinical herpes labialis or shedding of HSV without disease [Openshaw & Bennet, 1982], it is probable that the dental manipulation caused the HSV-1 reactivation, leading to development of facial palsy in the patient with herpes labialis.

HSV-1 reactivation is not always accompanied with an antibody response, and a serological assay is not useful for detecting HSV-1 reactivation [Cesario, et al., 1969]. In the present study, only 3 of 42 patients had significant changes in their anti-HSV IgG antibody titer or an increased IgM antibody titer, indicating a recent HSV-1 infection. Reactivation of HSV-1 into saliva, however, was detected by PCR in 40% of the HSV-seropositive patients with acute-phase Bell's palsy. Using the nested-PCR method described in the present study, HSV-1 reactivation can be detected within 2 days. It is reasonable to treat HSV-1-positive patients with an anti-viral agent because the virus is replicating in these patients. Recently, Adour et al. [1996], based on the hypothesis that HSV reactivation is the most probable cause of Bell's palsy, reported the results of a double-blind study: Treatment with acyclovir plus prednisone had a better outcome than prednisone-only therapy for patients with Bell's palsy. Although serological data concerning herpes infection were not described in the report, it is possible to speculate that patients with HSV reactivation, as well as those with varicella-zoster virus reactivation without vesicles (zoster sine herpete) were included in the study, and that such patients responded to the acyclovir treatment. Further studies based on the PCR and serological data are needed to evaluate the effect of the anti-viral therapy in patients with Bell's palsy.

In conclusion, it was demonstrated that shedding of HSV-1 into saliva was detected by PCR in 40% of HSV-seropositive patients with Bell's palsy during the acute phase of the disease. Although we must further analyze whether facial palsy is caused by direct injury to the facial nerve by reactivated HSV-1 or by a virus-associated immune-mediated mechanism, our data may add further evidence to the hypothesis that reactivation of HSV-1 is implicated in the pathogenesis of Bell's palsy. In addition, it was shown that PCR is useful for the early diagnosis of HSV-1 reactivation in patients with Bell's palsy.

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## REFERENCES


Adour KK, Bell DN, Hilsinger RLJ (1975): Herpes simplex virus in idiopathic facial paralysis (Bell Palsy). Journal of American Medical Association 233:527–530.

Adour K, Ruboyianes J, Von Doersten P, Byl F, Trent C, Quesenberry C, Hitchcock T (1996): Bell's palsy treatment with acyclovir and prednisone compared with prednisone alone: A double-blind, randomized, controlled trial. Annals of Otology Rhinology Laryngology 105:371–378.

Baringer JR, Swoveland P (1973): Recovery of herpes-simplex virus from human trigeminal ganglions. New England Journal of Medicine 288:648–650.

Baringer JR (1996): Herpes simplex virus and Bell palsy. Annals of Internal Medicine 124:63–65.

Cesario TC, Poland JD, Wulff H, Chin TDY, Wenner HA (1969): Six years experience with herpes simplex virus in a children's home. American Journal of Epidemiology 90:416–422.

Djupesland G, Berdal P, Johannessen TA, Degré M, Stien R, Skrede S (1976): Viral infection as a cause of acute peripheral facial palsy. Archives of Otolaryngology 102:403–406.

Furuta Y, Takasu T, Sato KC, Fukuda S, Inuyama Y, Nagashima K (1992a): Latent herpes simplex virus type 1 in human geniculate ganglia. Acta Neuropatholgica (Berl) 84:39–44.

Furuta Y, Takasu T, Fukuda S, Sato-Matsumura KC, Inuyama Y, Hondo R, Nagashima K (1992b): Detection of varicella-zoster virus DNA in human geniculate ganglia by polymerase chain reaction. Journal of Infectious Diseases 166:1157–1159.

Kameyama T, Sujaku C, Yamamoto S, Hwang CBC, Shillitoe EJ (1988): Shedding of herpes simplex virus type 1 into saliva. Journal of Oral Pathology 17:478–481.

Lynas C, Cook SD, Laycock KA, Bradfield JWB, Maitland NJ (1989): Detection of latent virus mRNA in tissues using the polymerase chain reaction. Journal of Pathology 157:285–289.

McCormick DP (1972): Herpes-simplex virus as cause of Bell's palsy. Lancet 1:937–939.

McGeoch DJ, Dalrymple MA, Davison AJ (1988): The complete DNA sequence of the long unique region in the genome of herpes simplex virus type 1. Journal of General Virology 69:1531–1574.

Morgan M (1992): Facial palsy and infection: The unfolding story. Clinical Infectious Diseases 14:263–271.

Mulkens PSJZ, Bleeker JD, Schröder FP (1980): Acute facial paralysis: A virological study. Clinical Otolaryngology 5:303–310.

Murakami S, Mizobuchi M, Nakashiro Y, Doi T, Hato N, Yanagihara N (1996): Bell palsy and herpes simplex virus: Identification of viral DNA in endoneurial fluid and muscle. Annals of Internal Medicine 124:27–30.

Openshaw H, Bennet HE (1982): Recurrence of herpes simplex virus after dental extraction. Journal of Infectious Diseases 5:707–713.

Spruance SL (1994): Bell palsy and herpes simplex virus. Annals of Internal Medicine 120:1045–1046.

Takasu T, Furuta Y, Sato KC, Fukuda S, Inuyama Y, Nagashima K (1992): Detection of latent herpes simplex virus DNA and RNA in human geniculate ganglia by polymerase chain reaction. Acta Otolaryngologica (Stockh) 112:1004–1011.

Tateishi K, Toh Y, Minagawa H, Tashiro H (1994): Detection of herpes simplex virus (HSV) in the saliva from 1,000 oral surgery outpatients by the polymerase chain reaction (PCR) and virus isolation. Journal of Oral Pathology and Medicine 23:80–84.

Vahlne A, Edström S, Arstila P, Beran M, Ejnell H, Nylén O, Lycke E (1981): Bell's palsy and herpes simplex virus. Archives of Otolaryngology 107:79–81.